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Office Microscopy in the Diagnosis of Infectious Diseases

SUMMARY

Microscopy of clinical specimens is a rapid and inexpensive method for the presumptive diagnosis of certain infectious diseases. Early diagnosis permits the physician to initiate appropriate therapy without waiting for culture results. Examination of the Gram-stained smear gives information on the inflammatory response, as well as the bacteria involved. Accurate interpretation of the smear, however, requires some training and experience. This article describes the microscopic methods commonly used in the laboratory and their application to office practice. (*Can Fam Physician* 1988; 34:379–383.)

RÉSUMÉ

L'étude microscopique de spécimens cliniques constitue une méthode rapide et peu coûteuse pour préciser le diagnostic de certaines infections. Un diagnostic précoce permet au médecin de débuter le traitement approprié sans attendre les résultats de la culture. L'examen par la coloration de Gram permet d'obtenir des informations sur la réponse inflammatoire et sur le type de bactérie en cause. Toutefois, l'interprétation précise du frottis exige un certain niveau de formation et d'expérience. Cet article décrit les méthodes microscopiques les plus fréquemment utilisées au laboratoire et leur application au bureau.

Key words: office microscopy, infectious diseases, rapid diagnosis

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THE DIAGNOSIS of infectious diseases has traditionally been carried out in a centralized microbiology laboratory, by means of microscopy and culture techniques. Recently antigen detection systems have been introduced, and they provide a rapid means of diagnosing specific infections. Commercially available kits can be used for the diagnosis of a variety of infections. Many of these tests are simple and can be performed in a physician's office. The availability of these tests, along with the increasing demands for cost-effectiveness in medical care, has resulted in a trend towards the performance of more diagnostic tests in the clinical setting. Rapid testing enables the physician immediately to correlate the results with the clinical findings and to start early antimicrobial therapy where appropriate. In some instances the need for expensive culture may be avoided. All these factors will directly increase the cost of medical care and, by allowing the patient to resume normal productivity earlier, will result in indirect cost savings.

Microscopy of clinical specimens is a simple and rapid method of screening for evidence of an infection and for making a presumptive diagnosis of an infectious disease or a presumptive identification of an infectious agent. Microscopy is cheaper than the antigen-detection systems and has a broader range of application. In a recent study conducted in the United

States, 97% of family physicians surveyed had access to an office laboratory.[1] Over 90% of these physicians had microscopes in their laboratories. The most frequently performed test was microscopic examination of the urine sediment.

We describe here a practical approach to the use of office microscopy in the diagnosis of infectious diseases.

Microscopic Methods

Fresh clinical specimens may be examined microscopically after preparation of a stained smear or unstained as a wet mount. The Gram stain is the classical stain used in clinical microbiology. It is a differential stain consisting of two dyes added sequentially to the smear with an intermediate decolourization procedure. Bacteria which retain the first dye, crystal violet, are Gram-positive and appear purple. Gram-negative bacteria do not retain the first dye during decolourization, and so stain with the second dye, safranine, and appear pink. In a Gram-stained smear, bacteria are also distinguished by their shape (i.e., cocci or bacilli) and by their arrangement, clusters, chains or pairs. All stained smears are examined under oil immersion using a X100 objective. Gram-stained smears can be used for examining any clinical material, such as urine, pus, sputum, or cerebrospinal fluid.

Clinical specimens may also be examined unstained by means of bright field microscopy with an X40 objective. These preparations are usually used to detect pus cells in urine and parasites in fecal specimens or in vaginal discharge A drop of 10% potassium hydroxide solution is added to the preparation when the technologist is looking for fungal elements in skin scrapings.

Preparation of a Gram-stained smear

- Use a dry, clean, microscope slide. If the specimen is a fluid (e.g., urine, sputum, pus), place 1–2 drops on the slide and using a sterile dry swab, spread the specimen over an area approximately 1 cm × 2 cm. If the specimen has been collected on a swab, rub the swab over a similar area on the slide. (This swab cannot be sent for culture as it is potentially contaminated; a second swab must be collected if culture is required.) Mark the area of the smear on the under surface of the slide, using a wax pencil.
- Allow the smear to air dry, and then fix it by passing the slide through the blue flame of a Bunsen burner 3–4 times. (Do not overheat the slide.) Allow the slide to cool. Heating causes the material to stick to the slide and kills the bacteria.
- Place the slide on a stain rack over a sink and flood with crystal violet solution.
- After one minute wash the slide with water, and cover the smear with Gram's iodine. Allow it to remain for approximately one minute.
- Wash with tap water and apply decolourizer (95% ethyl alcohol or acetone alcohol which consists of equal parts of 95% alcohol and acetone) for approximately 5–10 seconds; wash and repeat the procedure till no more blue colour runs off the slide. The number of times this is done will depend on the thickness of the smear, as thick smears will require longer decolourizing.
- Rinse with water and apply the counterstain safranine for 30 seconds. Rinse with water and gently blot dry.

Control smears of known bacteria should be done along with the specimen if the examiner is inexperienced. An experienced examiner will be able to judge the quality of the stain by the appearance of the neutrophils and the bacteria. The smear should first be screened using a x10 objective (low-power lens) for an area of abundant neutrophils, and then examined by means of the oil-immersion lens (x100 objective). The numbers of bacteria present, their morphology, and their relationship to the neutrophils should be noted.

Wet mount

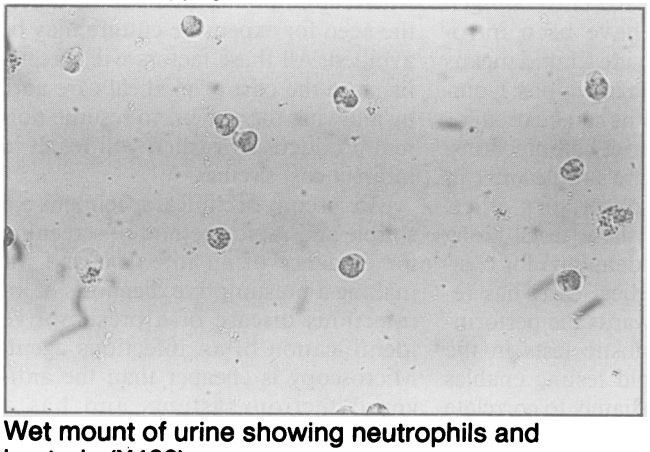
One to two drops of the specimen are applied directly to the slide. If the material is too thick, a drop of normal saline is added to dilute it. A coverslip is gently placed over the material, and any excess liquid is blotted from the sides with a tissue. The slide is examined with the use of an X40 objective and a low-intensity light.

KOH preparation

One to two drops of 10% potassium hydroxide (KOH preparation) are placed on the slide, and the specimen is added. A coverslip is placed over the material, and the slide is examined after a wait of a few minutes which allows the material to "settle".

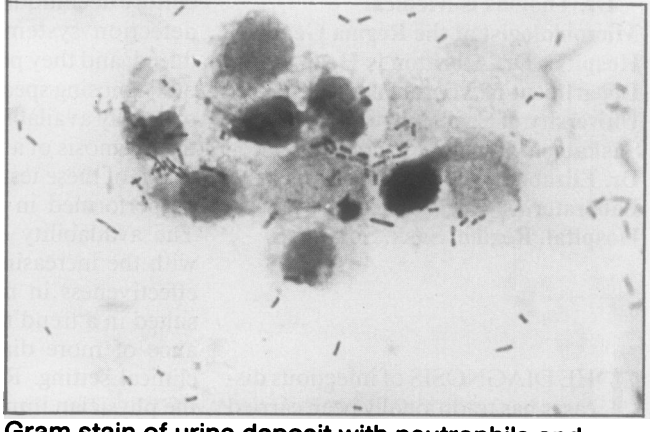
All slides are treated as potentially infectious and discarded in a container for contaminated waste.

Figure 1
Urine: Wet Mount



Wet mount of urine showing neutrophils and bacteria (X400).

Figure 2
Urine Gram Stain



Gram stain of urine deposit with neutrophils and gram-negative bacilli (X1000).

Application of Microscopy to Clinical Specimens

Microscopy is most applicable in the diagnosis of urinary tract infections, genital infections, bacterial pneumonia, and pyogenic infections.

Urinary tract infections (UTI)

Symptoms of UTI are among the most common complaints of patients presenting to physicians. Findings on urine microscopy can be correlated to culture results of a significant bacteriuria ($>10^5$ organisms/mL of urine)[2] and therefore help the physician to decide whether to treat the patient empirically while waiting for culture results.

Microscopy may be done on centrifuged or uncentrifuged urine by examining an unstained or stained preparation.

Examination of an unstained uncentrifuged urine sample. By means of a Pasteur pipette, a drop of well-mixed urine is placed on a glass slide (see Figure 1). The urine is covered with a glass coverslip and, with the use of an X40 objective and low-intensity light, the preparation is examined for neutrophils and organisms. This method, though very easy to perform, has low sensitivity and specificity.[3]

Examination of stained uncentrifuged urine. With a Pasteur pipette, two drops of well-mixed urine are placed on a microscopic slide and spread over an area of 1 cm × 2 cm (see Figure 2). After the smear has dried, it is heat fixed, Gram-stained, and examined with the use of an X100 oil-immersion objective. A minimum of five fields are examined. The presence of >2 organisms/oil-immersion field (OIF) corresponds to a specificity of 95%, and any organisms seen represent a sensitivity of 95% for detecting significant bacteriuria.[3] This method is more sensitive and specific when compared to unstained urine, but requires additional time for the Gram stain.

Examination of stained centrifuged urine. Ten mL of fresh urine are centrifuged for five minutes at 2500 rpm, and the sediment is used to make a Gram-stained smear. The presence of > 1 organism per OIF corresponds to a sensitivity of 95% and >5 organisms/OIF represent a specificity of 95% at the 10^5 organisms/mL level.[3] This method is more reliable and reproducible than either of the other two methods, but takes more time and requires an office centrifuge.

Genital infections

The use of the Gram stain in the diagnosis of gonorrhea is well established. The presence of intracellular Gram-negative diplococci in a smear of urethral discharge from a symptomatic male is a sufficient basis on which to make a presumptive diagnosis of gonorrhea. In females, however, the sensitivity of Gram-stained smears in detecting gonorrhea drops to as low as 55%.[4] Furthermore, the presence of saprophytic *Neisseria* can result in a false-positive result (See Figure 3).

Gram-stained smears of vaginal discharge are helpful in the diagnosis of *Candida* infections. *Candida* appear as single budding yeast cells or as mycelia with pseudohyphae (see Figure 4). The pseudohyphae are attached and have constrictions at the ends giving them the appearance of links of sausage.

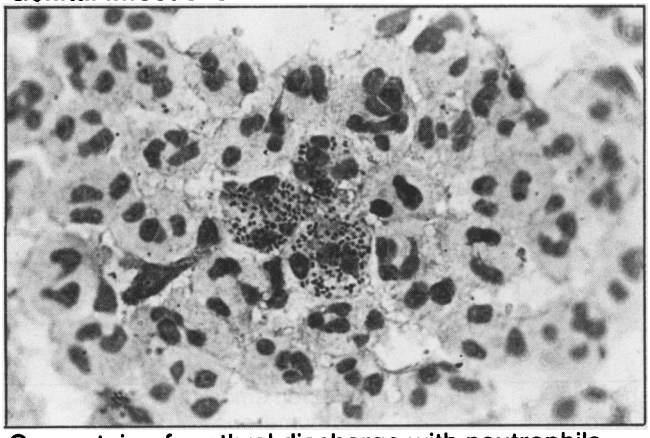
Candidal vaginitis may also be diagnosed by examining a wet mount or KOH preparation of the vaginal discharge. Again *Candida* may be seen as yeast or mycelial forms (see Figure 5).

Wet mounts of vaginal or urethral discharge, or a sediment of freshly passed urine can be examined for *Tri-*

Table 1
Microscopy Methods and Their Application

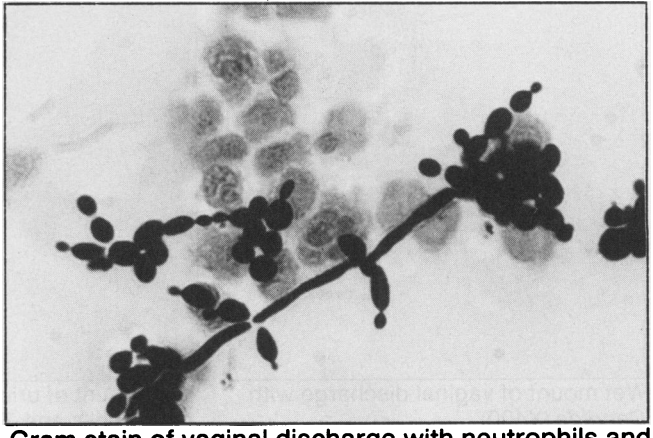
Method	Application
Gram-stained smear	1. Urinary tract infections 2. Genital infections 3. Pneumonia 4. Abscess
Wet mount	1. Urinary tract infections 2. Genital infections
KOH preparations	1. Dermatophytes 2. Vaginal candidiasis

Figure 3
Genital Infections



Gram stain of urethral discharge with neutrophils and gram-negative diplococci (X1000).

Figure 4
Genital Infections: Gram Stain



Gram stain of vaginal discharge with neutrophils and *Candida* (X1000).

chomonas vaginalis. These parasites have no cyst form, but appear as pear-shaped trophozoites with a characteristic jerky movement and have an undulating membrane that extends halfway down the body (see Figure 6). Wet-mount examination has a 80%–90% positive predictive value when compared to culture in the diagnosis of infection in symptomatic women.[5] The predictive value in asymptomatic women is much lower.

There is still some uncertainty of the exact role of *Gardnerella vaginalis* as a causative agent in non-specific vaginitis or bacterial vaginosis. However, in a patient with symptoms and/or signs of an abnormal vaginal discharge in whom *C. albicans, T. vaginalis,* cervicitis, and cervical ectopy have been excluded, a wet-mount examination may be used to establish the diagnosis of *G. vaginalis* infection. Vaginal epithelial cells covered with coccobacilli to the extent that the edges have been obliterated are known as "clue cells", and the presence of four or more clue cells per high-power field are presumptively diagnostic of *G. vaginalis* infection.[6] Liberation of an amine-like fishy odour when vaginal secretion is mixed with a KOH solution (100 g/L) is also characteristic of this infection, as is a pH of >4.5 for vaginal secretions.[6]

Pneumonia

Sputum bacteriology for the detection of pathogens may be subject to error because of oropharyngeal contamination and improper collecting and transporting of the specimen. Most laboratories routinely screen sputa in order to check their suitability for culture. A Gram-stained sputum is examined, and only those with <25 squamous epithelial cells per low-power field and on equal or greater number of neutrophils are considered suitable for culture (see Figure 7).[7] This screen could be done in the office, and only those specimens meeting the criteria sent for culture.

In community-acquired pneumonias the preparation of a Gram stain of sputum, along with the taking of a chest X-ray and a WBC count, is essential initial procedure for evaluation. Depending on the presence of the predominant morphotype associated with several neutrophils in the Gram film, a presumptive diagnosis of the type of pneumonia is made, and appropriate therapy can be started. Rien and colleagues,[8] in a study of community-acquired pneumonia, identified pneumococcal pneumonias by the presence of >10 Gram-positive lancet-shaped diplococci/OIF (Figures 8 & 9). They found that with the use of these criteria, the accuracy of a Gram stain in identifying pneumococci in the sputum was 100% specific and 48% sensitive, and had a positive predictive value of 100%. Baigelman and colleagues[9] examined sputa from patients with chronic bronchitis. They found the presence of >12 *Haemophilus*-like, >8 pneumococcus-like, or >18 *Neisseria*-like organisms/ OIF was often associated with an acute exacerbation of the disease.

Other infections

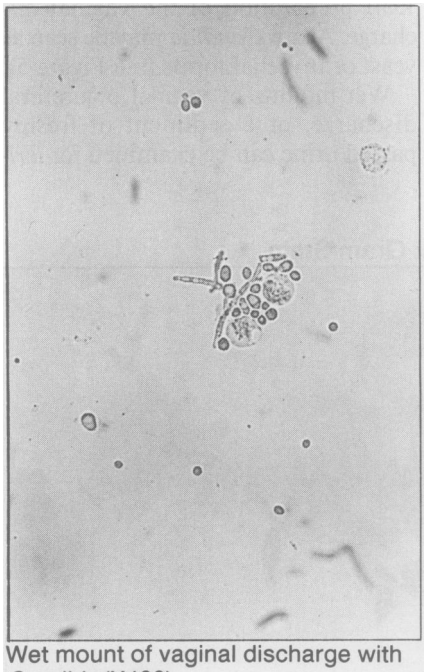
A Gram stain may be used in the diagnosis of any pyogenic infection from a variety of body sites. It is especially useful in examining pus from freshly drained abscesses, as differentiation of pure staphylococcae from a mixed anaerobic infection has important therapeutic implications (Figure 10).

The KOH preparation is also widely used for diagnosing dermatophyte infections. The presence of hyphae in the preparation indicates need for treatment.

Interpretation of Test Results

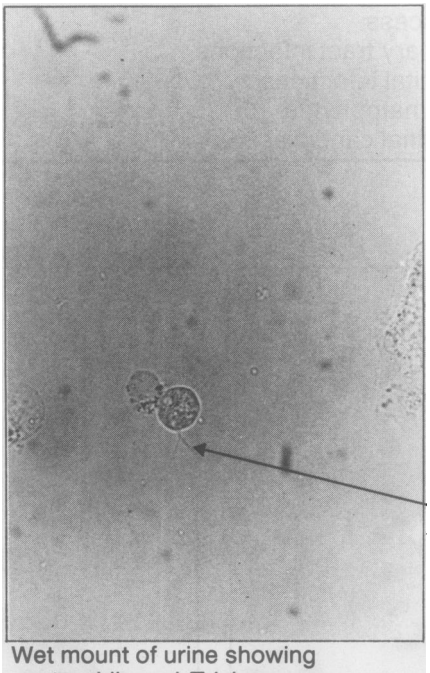
When evaluated on a sufficiently large sample, all tests are less than 100%

Figure 5
Genital Infections: Wet mount



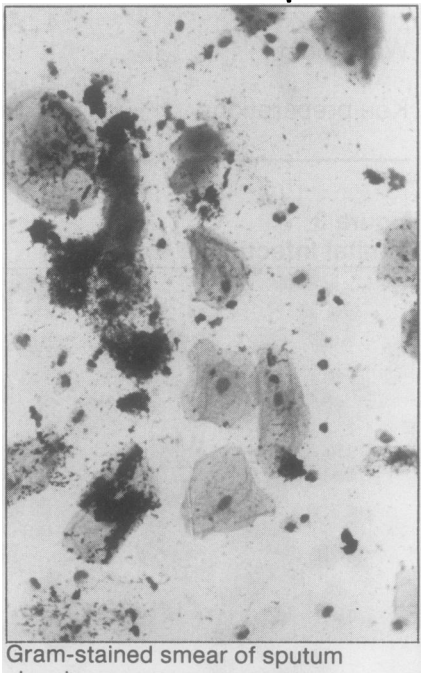
Wet mount of vaginal discharge with *Candida* (X400)

Figure 6
Wet Mount of Urine



Wet mount of urine showing neutrophils and *Trichomonas vaginalis flagellae* (X400).

Figure 7
Gram-Stained Smear of Sputum



Gram-stained smear of sputum showing numerous squamous epithelial cells and few neutrophils (X100).

sensitive and specific. Thus all tests, including microscopy, produce false-positive and false-negative results. The contribution of false-positive and false-negative results to the predictive value of a positive or negative test result depends strongly on the pre-test probability or prevalence of disease.[10] The utility of a test must thus be established in each clinical setting, and physicians using microscopy or other office tests should determine the positive and negative predictive value of the tests in their own practice setting, using culture results or other definitive tests as the "gold standard".

Conclusions

Microscopy is a rapid method used in diagnosing infection. It is simple enough so that it can be done in the physician's office and is cost effective. The cost of preparing and examining a Gram-stained smear in a clinical microbiology laboratory by a trained technologist is $1.28, and that of a wet preparation is $0.48. The costs of these procedures will be considerably less in a physician's office when the salary of a trained technologist is eliminated.

Microscopy has its limitations and should be used as a presumptive diagnostic procedure and not as a substitute for more definitive culture and sensitivity testing of micro-organisms. The test, like any other, is limited by the quality of the specimen and the materials used. In particular the microscope used should be in good working order, and quality control of reagents and procedures performed regularly. The person reading the smears should be familiar with the morphology and staining characteristics of bacteria and host cells in order to interpret the findings, and should participate regularly in continuing training and external evaluation of proficiency. Finally, the hospital microbiology laboratories should be prepared to offer assistance to family practitioners who wish to perform these tests. This assistance would help ensure that appropriate tests are being done at an acceptable level of proficiency and thus optimize patient care. ◉

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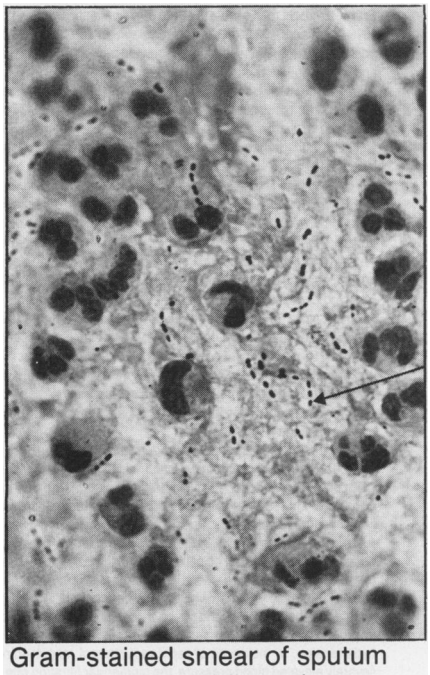
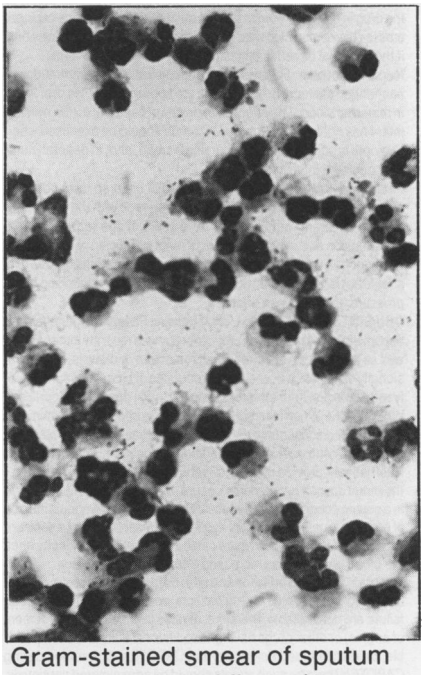
Figure 8
Gram-Stained Smear of Sputum



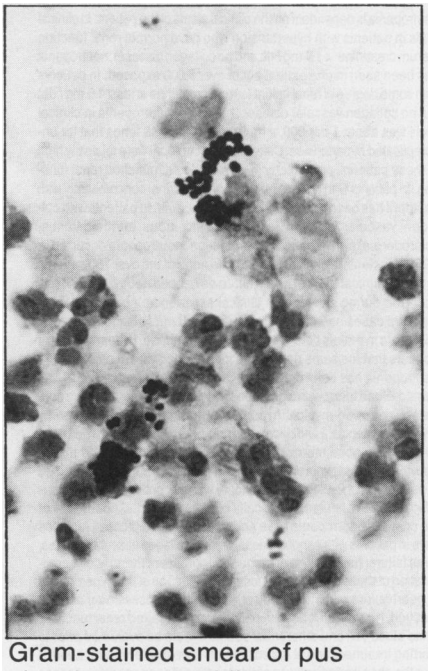
Gram-stained smear of sputum showing neutrophils and capsulate gram-positive diplococci (X1000).

Figure 9
Gram-Stained Smear of Sputum



Gram-stained smear of sputum showing neutrophils and gram-negative cocco-bacilli (X1000).

Figure 10
Gram-Stained Smear of Pus



Gram-stained smear of pus showing neutrophils and gram-positive cocci in clusters (X1000).